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THE STRUCTURE, LIFE HISTORY, AND INTRA-GENERIC RELATIONSHIPS OF *PARAMECIUM CALKINSI*, SP. NOV.

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(SEVEN FIGURES.)

The term *Paramecium* was coined by Dr. John Hill of London in 1752 for a group of animalcules comprising "four species," two of which probably include members of the genus *Paramecium* as recognized today.¹ Then O. F. Müller in 1773² named an organism *Paramæcium aurelia*, and in 1786,³ in the first comprehensive attempt to classify scientifically microscopic animals, definitely established the genus *Paramecium* and included under it five species. Unfortunately, however, without the slightest warrant etymological or otherwise, Müller changed the spelling of *Paramecium* to *Paramæcium*. Among the species which Müller distinguished are *Paramæcium aurelia* and *Paramæcium versutum*. The former apparently is Hill's "species 3" and either the present-day *P. aurelia* or *P. caudatum*. *Paramæcium versutum* possibly is Hill's "species 1" and apparently the present species *P. bursaria*. Müller's other paramecia have since been relegated to other genera.

Ehrenberg, in his great monograph on the Infusoria⁴ (1838), recognized eight species under the genus and returned to the spelling of Hill with the note "*Paramecium* ist richtiger als *Paramæcium*." Among Ehrenberg's species are two true paramecia: *Paramecium aurelia*, which he assumed to be the *P. aurelia* of Müller, and *P. caudatum*, which he distinguished from *P. aurelia* chiefly by the greater attenuation of the posterior end of the animal. Müller's species *P. versutum*, to which Focke in the meantime⁵ had given the name *Paramecium bursaria*, Ehrenberg placed under the genus *Loxodes* as *L. bursaria*.

¹ "History of Animals." London, 1752.

² "Vermium terrestrium et fluviatilium Historia." Havniae et Lipsiae, 1773.

³ "Animalcula Infusoria fluviatilia et marina." Havniae, 1786.

⁴ "Die Infusionsthierchen als vollkommene Organismen." Leipzig, 1838.

⁵ "Ueber einige Organisationsverhältnisse bei polygastrischen Infusorien und Rädertieren." *Isis*, 1836.

In 1841 Dujardin⁶ recognized only two species of *Paramecium*, *P. aurelia* and *P. caudatum*, and placed *P. versutum* of Müller (= *P. busaria* of Focke = *Loxodes bursaria* of Ehrenberg) in the genus *Bursaria*, though apparently with some hesitancy.

Claparède and Lachmann in their series of studies (1858–1860)⁷ distinguished eight species of *Paramecium*, including *P. putrinum*, a new species which is still recognized, *P. bursaria* and *P. aurelia*. They abolished *P. caudatum* with the remark "Le *P. caudatum* Ehr. n'en est certainement pas spécifiquement différent. C'est une simple variété qu'on pourrait même à bon droit considérer comme la forme typique de l'espèce."⁸

Thenceforth, for about thirty years, the name *Paramecium aurelia* was applied indiscriminately to both *P. aurelia* and *P. caudatum*. Most of the pioneer studies on conjugation, etc., were made, as we know now, on *P. caudatum*, though it was called *P. aurelia*. Then came the first hint which was to straighten out the matter. Maupas in 1883⁹ noted a difference in the micronuclear apparatus of certain paramecia which led him in 1888¹⁰ to announce definitely that the structure and number of the micronuclei is a distinctive character and thus on this basis to reestablish the two common species of *Paramecium*. Since 1889, when both Maupas¹¹ and Hertwig¹² studied the nuclear phenomena during conjugation of these forms, *P. aurelia* and *P. caudatum* generally have been regarded as distinct species, and the validity of this conclusion has been emphasized by Jennings and Hargitt,¹³ and by Woodruff¹⁴ using pedigree culture methods.

Studies on the conjugation phenomena of *P. bursaria* by

⁶ "Histoire naturelle des Zoophytes, Infusoires, etc." Paris, 1841.

⁷ "Études sur les Infusoires et les Rhizopodes," Geneva, 1858–1860.

⁸ *Ibidem*, part I., p. 265.

⁹ "Contributions à l'étude morphologique et anatomique des Infusoires ciliés," *Arch. de Zool. Exp. et Gen.*, (2), I., 1883, p. 660.

¹⁰ "Sur la multiplication des Infusoires ciliés," *Arch. de Zool. Exp. et Gen.*, (2), 4, 1888, p. 231.

¹¹ "Le rajeunissement karyogamique chez les ciliés," *Arch. de Zool. Exp. et Gen.*, (2), 7, 1889.

¹² "Ueber die Konjugation der Infusorien," *Abh. Kgl. bayr. Akad. d. Wiss.*, München, 2, Cl. 17, 1889.

¹³ "Characteristics of the diverse races of *Paramecium*," *Journ. Morph.*, 21, 1910.

¹⁴ "*Paramecium aurelia* and *Paramecium caudatum*," *Journ. Morph.*, 22, 1911.

Hamburger¹⁵ and of *P. putrinum* by Doflein¹⁶ apparently leave no question in regard to the validity of these species, though both species are remarkably similar.

In addition to *P. aurelia*, *P. caudatum*, *P. bursaria* and *P. putrinum*, which are accepted by Schewiakoff in his monographic survey of the holotrichous Infusoria,¹⁷ there must be mentioned *Paramecium trichium* described by Stokes¹⁸ in 1885, and *P. multimicronucleata* by Powers and Mitchell¹⁹ in 1910.

Stokes discovered *P. trichium* in the zoöglæa on an infusion of dead leaves. His description, in part, is as follows:

"It seems to be a distinct species, and one that can scarcely be mistaken for any known form, except possibly for *P. bursaria* (Ehr.) S. K., differing from the latter, however, conspicuously in form, especially in the apparently oblique curvature of the anterior extremity, in the absence of the truncation of the same part, the absence of the rapid and continuous circulation of the endoplasmic contents, and particularly the green coloration of the cortex and sarcode. The two contractile vesicles, instead of being placed one in each body-half, as in *P. bursaria*, are here anterior and close together, contracting quickly, the one beginning to again form almost before the completion of the other's systole. Trichocysts are very abundant, and are so arranged that they seem to elevate the cuticular surface into the minute hemispherical bosses that cover the entire body. When forcibly extruded through the influence of the glycerole of tannin, the distal end of each, for about one tenth of the entire length, is conspicuously thickened, so that the trichocyst seems to be supplemented by the addition of a minute pyramid."

Schewiakoff considers *P. trichium* to be identical with *P. putrinum*, but if Stokes' description is accurate there is no warrant for this conclusion. Certainly further data are needed before a final answer can be given.

¹⁵ "Die Konjugation von *Paramecium bursaria* Focke," *Arch. f. Protistenk.*, 4, 1904.

¹⁶ "Lehrbuch der Protozoenkunde," 2 Auflage, Jena, 1909.

¹⁷ "Organization et classification des infusoires *Aspirotricha* (*Holotricha auctorum*)," *Mem. Acad. Imp. Sci.*, St. Pétersbourg, Classe d. Sci. Phys. et Math., (8), 4, 1896.

¹⁸ "Some New Infusoria," *Amer. Naturalist*, XIX., 1885.

¹⁹ "A New Species of *Paramecium* (*P. multimicronucleata*) Experimentally Determined," *BIOL. BULL.*, 19, 1910.

P. multimicronucleata, as the name indicates, characteristically has many small micronuclei, usually from six to nine, the structure of which clearly resembles that of the micronuclei of *P. aurelia*. The authors' figures show a general cell-form which is more or less intermediate between that which is typical for *P. aurelia* and for *P. caudatum*. Powers and Mitchell emphasize the persistence of the specific characters after conjugation. It is an interesting form which warrants further study, especially in view of the fact that we now know that reduplication of micronuclei occurs during endomixis.

In January, 1919, I isolated a ciliate, from an infusion of fresh water and vegetable debris collected at New Haven, which, though undoubtedly a *Paramecium*, showed characters that distinguished it from any of the species hitherto described. It has now been extensively studied in pedigree cultures for more than a year and it has bred true. I have, therefore, definitely designated it a new species, and named it *Paramecium calkinsi*²⁰ in recognition of the fact that Professor G. N. Calkins of Columbia University introduced students of the Infusoria to exact daily isolation pedigree culture methods. *Paramecium calkinsi* presents an interesting composite picture of characters of *P. aurelia*, *P. bursaria*, *P. putrinum* and *P. trichium*, with certain distinctive ones of its own.

The general body form of *Paramecium calkinsi* is more similar to that of *P. bursaria*, *P. putrinum* and *P. trichium* than to that of *P. aurelia* and *P. caudatum*. Indeed, at first glance, one is reminded of *P. bursaria*, and further study suggests *P. putrinum* and *P. trichium* because individuals in different stages of nutrition and dividing at various rates exhibit quite a different appearance. The form which is most characteristic—in fact diagnostic—when the living cells are studied day after day in pedigree cultures is given somewhat diagrammatically in Figs. 1 to 4.²¹

The cells of this pedigree culture of *P. calkinsi*, under favorable food conditions, average about 120 μ in length and 50 μ in

²⁰ L. L. Woodruff, "*Paramecium calkinsi*, sp. n.," *Proc. Soc. Exp. Biol. & Med.*, Feb., 1921.

²¹ Drawings made by Miss J. E. Lovett of this Laboratory.

breadth. In other words, the length is about the same as that of many races of *P. aurelia*, but the breadth is proportionally

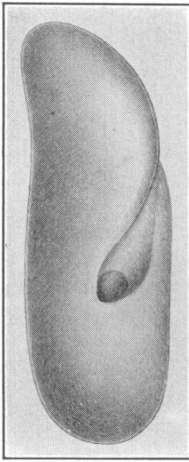


FIG. 1.

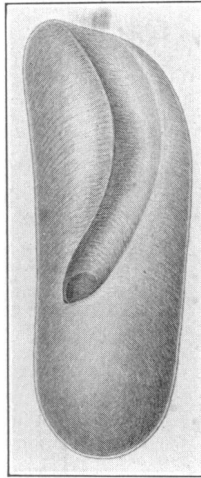


FIG. 2.

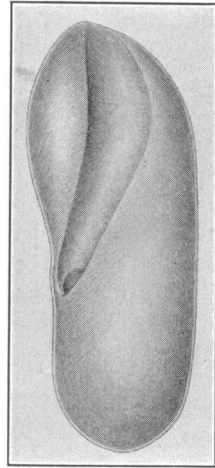


FIG. 3.

FIGS. 1-3. *Paramecium calkinsi*, sp. nov.

greater and the broadest part is anterior, whereas it is posterior to the middle in *P. aurelia*. But, of course, no emphasis can be placed on size relations since it is probable that when *P. calkinsi* from other localities is studied, racial size differences will be found to occur.

The ciliary apparatus of the new species affords no striking characteristic and an undulating membrane is present in the gullet as in all paramecia. The trichocyst apparatus is particularly well developed. The trichocysts are relatively evenly distributed in the cortical layer of protoplasm, except at the anterior end where they are more closely crowded together. The surface of the cell in profile presents, upon careful study, a slightly crenulated outline, each of the tiny elevations representing the position of a trichocyst and the depressions between affording the points of origin of the cilia. The structure of the trichocysts as they appear in situ is shown in Fig. 4. Exploded and detached examples appear in Fig. 5.

It will be noted that the trichocyst apparatus of *P. trichium*, as described by Stokes, and which he emphasized in naming the organism, agrees closely with that in the new species. The one

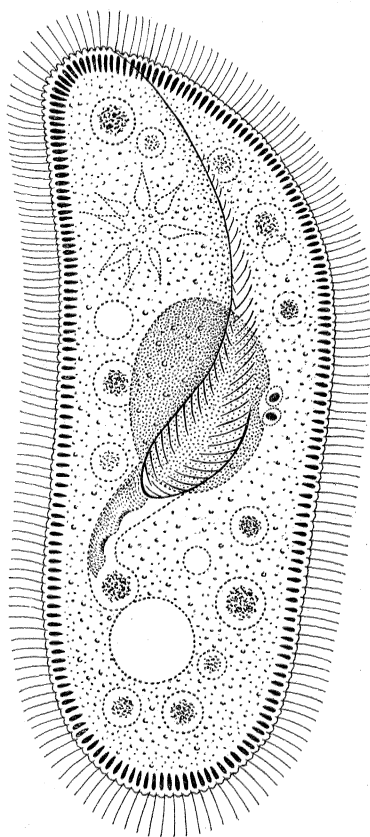


FIG. 4. *Paramecium calkinsi*, sp. nov.

essential difference is the form of the exploded trichocysts, which in *P. calkinsi* have almost the identical structure of those in *P. caudatum* as described by Khainsky.²²

The endoplasm of the new species is characteristically more vacuolated than that of *P. aurelia* or *P. caudatum* when all are bred in the same culture medium. On the other hand it is by no means so fluid as in *P. bursaria*. It is possible to distinguish almost at a glance the new species from the others, merely by the cytoplasmic appearance, when all are mingled together in the same culture.

The contractile vacuoles, as the figure shows, are two in number, and agree closely in position and form with those typical of *P. aurelia*, *P. caudatum*, and *P. bursaria*. Herein

is a striking difference from *P. putrinum* with one contractile vacuole at the anterior end and *P. trichium* in which two contractile vacuoles are situated close together near the anterior end.

The macronucleus presents no diagnostic characteristics, though, as the figure shows, in the culture under consideration it usually is proportionately larger in size than it is in the well-known species of *Paramecium*. Little emphasis, however, can

²² A. Khainsky, "Zur Morphologie und Physiologie einiger infusorien," *Arch. f. Protistenk.*, 21, 1910.

be placed on this as the size relations vary in all races of all species under different conditions. But I have never seen the macronucleus of *P. calkinsi* as small as Stokes figured it for *P. trichium*.

The micronuclear apparatus is almost identical with that of *P. aurelia* and in marked contrast with that of the other species (see Figs. 4 and 6). In the first place, there are characteristically

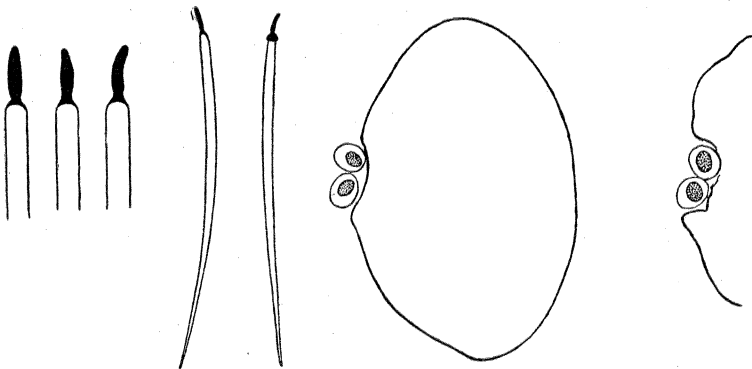


FIG. 5.

FIG. 6.

FIG. 5. 'Exploded' trichocysts of *Paramecium calkinsi*.

FIG. 6. Micronuclear apparatus of *Paramecium calkinsi*. Left, from a total preparation; right, from a section. Camera lucida sketches.

two micronuclei situated in or near a slight depression of the macronucleus. It has not been possible in every case to discover two micronuclei but there can be no doubt that two are typical of the vegetative cells. And, of course, as in all paramecia, the position of the micronuclei varies considerably in different individuals. Sometimes the macronuclear depression is on one side and sometimes on the other side. Frequently there are two tiny depressions each with a micronucleus. Again, the micronuclei may be at a considerable distance from the macronucleus, though not in mitosis.

In the second place, the relative size and the structure of the micronuclei are essentially similar to those of *P. aurelia*. The chromatin of the resting micronuclei is clumped to form an endosome which is surrounded by an 'achromatic' layer enclosed by a distinct nuclear membrane.

The general behavior as well as the 'avoiding reaction,' etc., of *P. calkinsi* is very similar to *P. caudatum* and *P. aurelia*, though the broad and slightly curved anterior end of *P. calkinsi* renders the revolutions on its long axis and its spiral path somewhat more conspicuous than in the more common species of *Paramecium*.

Paramecium calkinsi appears to be adapted to essentially the same environmental conditions as *P. aurelia* and *P. caudatum*. A series of experiments in which flasks of hay infusion were seeded with both *P. calkinsi* and *P. aurelia* showed that, as a rule, one and not both species attained a maximum development in the same flask. Usually within a few days there was a heavy growth of one and only a few struggling survivors, or none at all, of the other species. There was no indication that one was more hardy than the other under the conditions of the experiment; success or failure to dominate the culture apparently depended on which form 'got the start,' so to speak. It seems probable that the excretion products of the two forms are mutually detrimental and that this is the determining factor in the comparative incompatibility of the two species.²³ This point is being investigated.

The rate of reproduction of the new species, as exhibited in pedigree cultures throughout nearly a year, shows no factors which distinguish it from *P. aurelia*, *P. caudatum* or *P. bursaria*, which I have studied under similar conditions, since it is well known that characteristic division rates obtain in different races of the same species. The main pedigree culture of *P. calkinsi* was carried from January 30 to November 20, 1920, when it died out at the 321st generation (Fig. 7). During all this period a constant culture medium of standard beef extract was employed, and during certain experiments the temperature was maintained, by a thermostat, practically constant at 24° C. The race is now thriving in mass cultures which were seeded from the pedigree lines. The animals are apparently in as healthy condition as ever.

²³ L. L. Woodruff, "The Effect of Excretion Products of Infusoria on the Same and on Different Species, with Special Reference to the Protozoan Sequence in Infusions," *Jour. Exper. Zool.*, 14, 1913.

Throughout the year's work every effort has been made to secure conjugation and encystment in the mass cultures, though without result. Furthermore, an intensive study, involving the making of more than a thousand permanent preparations of pedigree animals at all stages in the life of the culture,²⁴ has failed to reveal any indications of endomixis. Therefore, it can be stated positively that endomixis has not occurred in this culture, although there are fluctuations of the division rate somewhat similar to those associated with endomixis in *P. aurelia* and *P. caudatum*.²⁵ This point will be considered in detail in another paper.

As is well known, the diet of *Didinium nasutum* is almost exclusively confined to paramecia, and it has not been possible to cultivate successfully the former without this food. An experiment extending through several months showed that *Didinium* thrives on *P. calkinsi* just as well as on *P. aurelia*—further evidence that the new species is a *Paramecium*!

From the foregoing description of the structure and life history of *P. calkinsi* it is apparent that this

²⁴ I am indebted to Miss Hope Spencer of the Osborn Laboratory for assistance in carrying out this work.

²⁵ Woodruff and Baitzell, "Rhythms in the Reproductive Activity of Infusoria," *Jour. Exper. Zool.*, 11, 1911. Woodruff and Erdmann, "A Normal Periodic Reorganization Process without Cell Fusion in *Paramecium*," *Jour. Exper. Zool.*, 17, 1914. Erdmann and Woodruff, "The Periodic Reorganization Process in *Paramecium caudatum*," *Jour. Exper. Zool.*, 21, 1916.

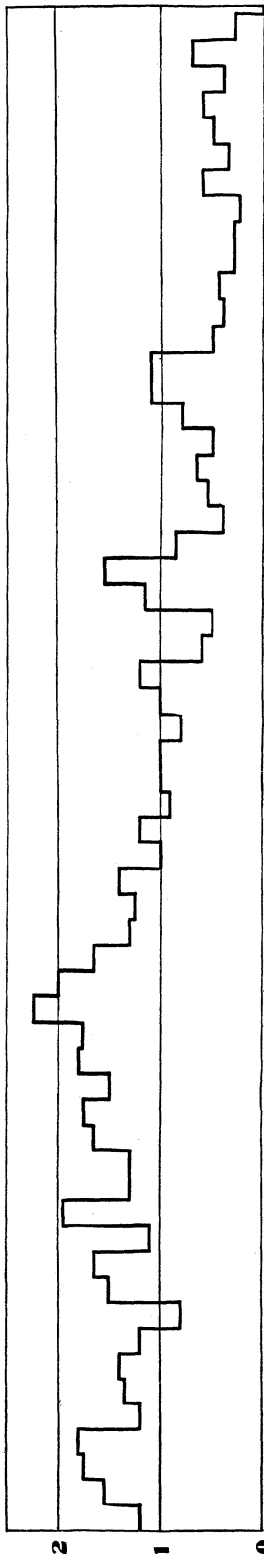


FIG. 7. Graph of the division rate of the main pedigree culture of *Parametium calkinsi* from January 30 to November 20, 1920. The ordinates represent the average daily rate of division of the four lines of the culture, again averaged for five-day periods.

species presents a complex mosaic of the characters of the other members of the genus²⁶—indeed the intrageneric relationships of the new species are its chief interest.

A survey of the six species of *Paramecium* under discussion shows that they fall into two quite clearly defined groups which may be referred to as the 'aurelia group' and the 'bursaria group' respectively. The members of the 'aurelia group' (*P. aurelia*, *P. caudatum* and *P. multimicronucleata*) are characterized by a relatively long spindle- or cigar-shaped body; those of the 'bursaria group' (*P. bursaria*, *P. putrinum*, *P. trichium* and *P. calkinsi*) by a somewhat shorter and broader form, with a tendency, especially prominent in *P. bursaria*, toward a dorsoventral flattening.

Within each of these groups are found two general structural types of micronuclei. One type, which may be termed the 'caudatum type,' is exhibited by *P. caudatum* in the 'aurelia group,' and by *P. bursaria*, *P. putrinum* and apparently by *P. trichium* in the 'bursaria group.' Though Stokes' figure of the micronucleus of *P. trichium* leaves much to be desired, it clearly indicates that the single micronucleus is not of the 'endosome' type. The other type of micronucleus, the 'endosome' or 'aurelia type,' occurs in *P. aurelia* and *P. multimicronucleata* in the 'aurelia group,' and in *P. calkinsi* in the 'bursaria group.' All species with the 'caudatum type' characteristically possess one micronucleus, and those with the 'aurelia type,' two micronuclei, except *P. multimicronucleata* in which the number apparently varies from six to nine.

Paramecium calkinsi represents the 'aurelia type' of micronuclear apparatus in the 'bursaria group' of species. It is possible that future study may show that certain of the paramecia under discussion (e.g., *P. micronucleata*, *P. trichium*, *P. putrinum*) should be regarded as racial forms rather than distinct species, but I believe that the data presented above indicate beyond doubt that *Paramecium calkinsi* takes its place by *P. bursaria*, as *Paramecium aurelia* has by *P. caudatum*, as a distinct species.

²⁶ It is unnecessary to illustrate the cell form and micronuclear structure of the several species of *Paramecium* because figures are available in many of the monographs cited. Especial reference should be made to the figures given by Jennings and Hargitt, Schewiakoff, and Hamburger.

March 4, 1921.